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Minimizing Variations in Functionality of Whey Protein Concentrates from Different Sources*

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ABSTRACT

Enhancement in processing technology has improved the nutritional and functional properties of whey protein concentrates by increasing the content and quality of the protein, leading to their increased use in different food products. The extent of heat treatment affects the quality of the whey protein concentrate, and wide variation in product quality exists due to the various means of manufacture and from the whey product history from farm to factory. The study was carried out with 6 commercial whey protein concentrates with 80% protein (WPC80) to determine variations in physical properties, particle size and density, and functional properties—solubility, gel strength, foam volume, and stability. Significant differences were observed among all the products for every property compared. Particulate size was the most important determinant of functional characteristics. Larger particulate WPC80 had significantly higher fat content and were less soluble with poor foam stability; but narrowing the particle size distribution through sieving, minimized variations. We determined that sieving all products within the particle size distribution range of 100 to 150 microns minimized variation in physical composition, making functionality uniform. WPC80 from different manufacturers can be made to perform uniformly within a narrow functionality range by reducing the particle size distribution through sieving.
(**Key words:** whey protein, processing technology)

Abbreviation key: WPC80 = whey protein concentrate with 80% protein.

INTRODUCTION

The use of whey protein concentrates in formulating products is increasing due to the nutritional and health benefits attributed to these proteins. Whey protein concentrate types available range from 35 to 80% protein. The quality of various whey concentrates is rising due to recent improvements in methods for isolating and concentrating the proteins from whey derived from cheese manufacture (Chadan, 1997). Final processing steps include filtration techniques, separation technologies such as ultrafiltration, and reverse osmosis, and ion exchange continues to improve protein quality, but adds variability in functionality such as solubility and gelling properties. The quality and functionality of whey protein concentrates depend on the source of cheese and process history (Caric, 1994; Huffman and Harper, 1999). Many variables associated with whey protein production, including source farm practices, cheese production method, acid or rennet coagulation, and choice of processes (membrane filtration or ion exchange), and spray drying, affect functionality significantly, producing manufacturer to manufacturer as well as batch to batch differences (Hurley, 1990).

The composition of whey protein powder depends on the type of cheese manufactured, the culture used, and the processing conditions of the cheese (Schmidt et al., 1984; Hurley, 1990). Variation in the breed of the dairy cattle and the composition of the herd affect the protein level (Mehra et al., 1999). The extent of heat-induced aggregation of lactoglobulins affect functional properties such as solubility (Regester et al., 1992). Various chemical and physical reactions, such as reactions of amino acid side chains, can form cross links, resulting in denaturation of the proteins and subsequent reduction in solubility (Walstra et al., 1999). Classification of whey proteins depends on the severity of heat treatment; the less denatured, low-heat powders are generally higher in solubility and foaming, while the more denatured, high-heat powders are generally lower in solubility (Sullivan and O'Connor, 1971).

Variability in whey proteins and their functionality in products is a significant problem in formulating products. To maintain consistency, food manufacturers must rely on one manufacturer or are forced to blend products from multiple sources to achieve uni-

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Table 1. Proximate composition and physical properties of the whey protein concentrate with 80% protein as purchased.

Product	Moisture %	Protein %	Fat %	Ash %	Carbohydrate %	Particle size (microns)
A	4.9[a]	75.8[b]	2.7[ab]	2.8[d]	13.8[b]	262[bc]
B	3.9[b]	77.0[ab]	4.2[a]	3.1[c]	11.8[c]	301[b]
C	4.0[b]	77.5[a]	4.0[a]	2.6[e]	11.9[c]	240[c]
D	3.4[c]	76.8[ab]	1.9[b]	3.2[c]	14.7[a]	53[e]
E	3.6[c]	76.0[b]	3.6[a]	4.5[b]	12.3[c]	382[a]
F	3.9[b]	74.3[c]	3.1[ab]	4.8[a]	13.9[b]	192[d]

[a,b,c,d,e]Means within a column with the same letter are not significantly different.

formity or reduce the quantity used. The use of whey proteins could be increased greatly by reducing variability from batch to batch and from manufacturer to manufacturer (Anon, 2000). The goal of this study was to determine the variability in whey properties and functionality from 6 commercial suppliers and determine methods for reducing variability in spite of the differences in manufacturing.

MATERIALS AND METHODS

Whey protein concentrate was purchased from the following companies: The Milky Whey Inc. (Missoula, MT), Foremost Farms USA (Baraboo, WI), Arla Foods, Inc. (Union, NJ), Kerry Foods (Beloit, WI), and Proliant Inc (Ames, IA). All whey protein concentrates were low heat processed, contained approximately 80% protein (**WPC80**), and were intended for use in extruded snack food applications. Proximate composition of the WPC80 products was determined as purchased. The WPC80 products were analyzed as purchased and after sieving through 2 standard meshes: 100-mesh (150-micron opening), 140-mesh (106-micron opening), making 3 classes of WPC80 products each per sample. The experiment was replicated twice, and analyses were done in triplicate. Analysis of SAS covariance was used to identify differences in physical properties among the 6 products. Duncan's multiple range test was used for mean separation, and correlation coefficients were calculated. The SAS package was used (SAS Institute Inc., Cary, NC) in all cases. Significance of differences was defined as $P \leq 0.05$.

Moisture content was determined by the AOAC method 925.10 (AOAC, 2000). Approximately 1.5 g of WPC80 product was dried in a vacuum oven at 100°C overnight (AOAC, 2000).

Ash content was determined by AOAC method 923.03 (AOAC, 2000). Ash was determined from 3-g samples combusted in a muffler furnace at 550°C for 16 h (AOAC, 2000).

Fat content was determined using AACC method 30-25 (AACC, 1995). A 1-g sample of WPC80 product was placed in an Erlenmeyer flask. One milliliter of sulfuric acid and 4 mL of water were added to the flask, and mixed gently. After 60 min, the contents of the flask were transferred to a 60-mL separatory funnel using 25 mL of dichloromethane: methanol solution (1:1). After 15 min, the bottom layer was drained into a weighing pan and then evaporated. The amount of fat was calculated according to AACC (1995).

Protein content was determined using the LECO Protein Analyzer model FP2000 (LECO Corporation, St. Joseph, MI). A 0.2-g sample was placed in the sample holder and analyzed. Percent protein was calculated with the nitrogen conversion factor 6.38 for whey protein.

Particle size distribution was determined for the original product, and the sieved samples. Each sample was analyzed using the Accusizer Optical Particle Sizer model 770 (Particle Sizing Systems, Santa Bar-

Table 2. Proximate composition and physical properties of the whey protein concentrate with 80% protein (100 mesh).[1]

Product	Moisture %	Protein %	Fat %	Ash %	Carbohydrate %	Solubility %
A	6.2[a]	77.7[bc]	2.9	2.6[c]	10.6[c]	60.1[cd]
B	4.4[b]	77.5[c]	3.6	3.0[bc]	11.5[b]	65.2[ab]
C	4.2[b]	77.2[c]	3.1	2.5[c]	13.0[a]	59.9[de]
D	3.7[c]	83.6[a]	1.9	3.2[b]	7.6[d]	65.6[a]
E	4.2[b]	79.9[b]	3.1	4.4[a]	8.4[d]	57.2[e]
F	4.3[b]	74.7[d]	2.4	4.7[a]	13.9[a]	62.8[bc]

[a,b,c,d,e]Means within a column with the same letter are not significantly different.

[1]Particle size: 100 to 150 microns, except for D (54 microns).

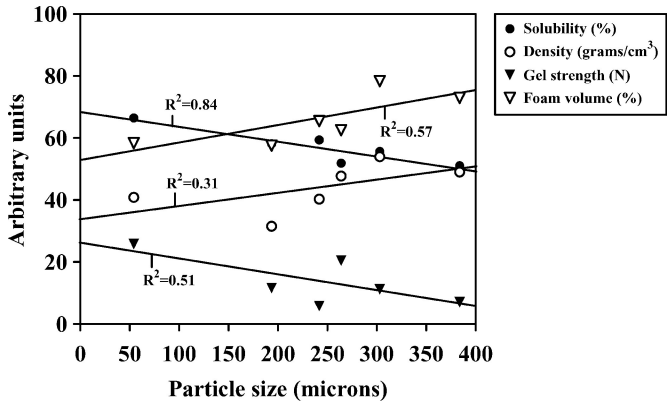

Figure 1. Physical properties of whey protein concentrate with 80% protein as purchased samples: solubility, density, gel strength and foam volume.

bara, CA). The particle size distribution of the samples was determined.

Particle density of the WPC80 samples was determined with an air pycnometer Horiba model VM-100 (Horiba Inc. Irvine, CA).

Gel Strength

Gel strength as described by Ju and Kilara (1998) was measured by Bloom determinations using a TA-XT2 Texture Analyzer. An 11% protein solution was made (3.2 g of dried sample mixed with 26.7 mL of deionized water and 3.3 mL of 0.03 *M* $CaCl_2$, and allowed to sit for 15 min. To initiate gelation, the sample was heated to 80°C for 30 min in a water bath, cooled in an ice bath for 15 min, and then stored overnight at 4°C. Gel strength was determined using a 0.5-inch analytical probe to a depth of 6 mm at the rate of 1 mm/s.

Protein Solubility and Denaturation

As described by Kilara (1984), 1.0 g of product was mixed with 90 mL of deionized water. The protein suspension was adjusted to pH 7 and then stirred at 125 rpm for 2 h. The suspension was then centrifuged for 20 min and decanted. The supernatant was freeze-dried overnight. The LECO Protein Analyzer model FP2000 (LECO Corporation, St. Joseph, MI) was used to analyze the solids from the freeze-dried supernatant for protein content. Protein solubility was calculated as described by Kilara (1984). (Percent protein denatured is the inverse of percent solubility).

Foam Volume and Stability

Foam volume and stability of the WPC80 products were determined using the method described in Phillips et al. (1990). Samples (2.3 g) of WPC80 product were mixed with 35 mL of deionized water and then heated to 60°C for 15 min. The slurry was whipped for 15 s in a Waring Laboratory Micronizer FPC70 (Waring Products Division, New Hartford, CT), and then transferred to a 100-mL graduated cylinder, where the foam volume was read initially, and for every 5 min for 1 h. Foam stability (foam capacity at specific time) over the 1-h period was calculated as described by Phillips et al. (1990).

Viscosity Analysis

Viscosity analysis of the pasting behavior of the WPC80 products was conducted with a Rapid Visco-Analyzer (RVA) model RVA-3D (FOSS North America, Eden Prairie, MN) equipped with Thermocline for Windows software. Pasting properties, a measure of WPC80 paste viscosity, were determined by RVA Application method no. 48, using a 28-g specimen, 13.5% wet basis. Specimens were stirred initially at 1000 rpm for 60 s followed by constant stirring at 320 rpm. At equilibrium, the specimens were heated from 50 to 80°C in 3 min, held at 80°C for 5 min, then cooled to 30°C in 4 min. Cold (initial), maximum (peak), trough, final, and breakdown viscosities were recorded (Parkes et al., 1998).

For scanning electron microscopy, WPC80 products (1 to 2 mg) were injected into 10 mm diameter. Spectrapor dialysis tubing (Spectrum Medical Industries, Inc., Los Angeles, CA) and equilibrated with a fixative solution containing 2% glutaraldehyde and 0.1 *M* imidazole HCl (pH 7.0) for 24 h. Samples were washed in imidazole buffer and dehydrated by exchange with 50% absolute ethanol for 24 h. The samples in the tubing were frozen in liquid nitrogen and fractured manually with the cooled blade of a surgical scalpel. Fractured fragments were thawed into absolute ethanol and critical point dried in liquid carbon dioxide. Dry fragments were glued to aluminum specimen stubs with colloidal silver paste (Electron Microscopy Sciences, Ft. Washington, PA) and coated by DC sputtering with a thin layer of gold for imaging in a model JSM 840A scanning electron microscope (JEOL USA, Peabody, MA), operated in the secondary electron imaging mode. Digital images were collected with an Imix workstation (Princeton Gamma-tech, Princeton, NJ). Image analysis of digital images (fast Fourier transformation) was done as described earlier (Cooke et al., 1995) to resolve possible differences in topographical features of the different whey samples.

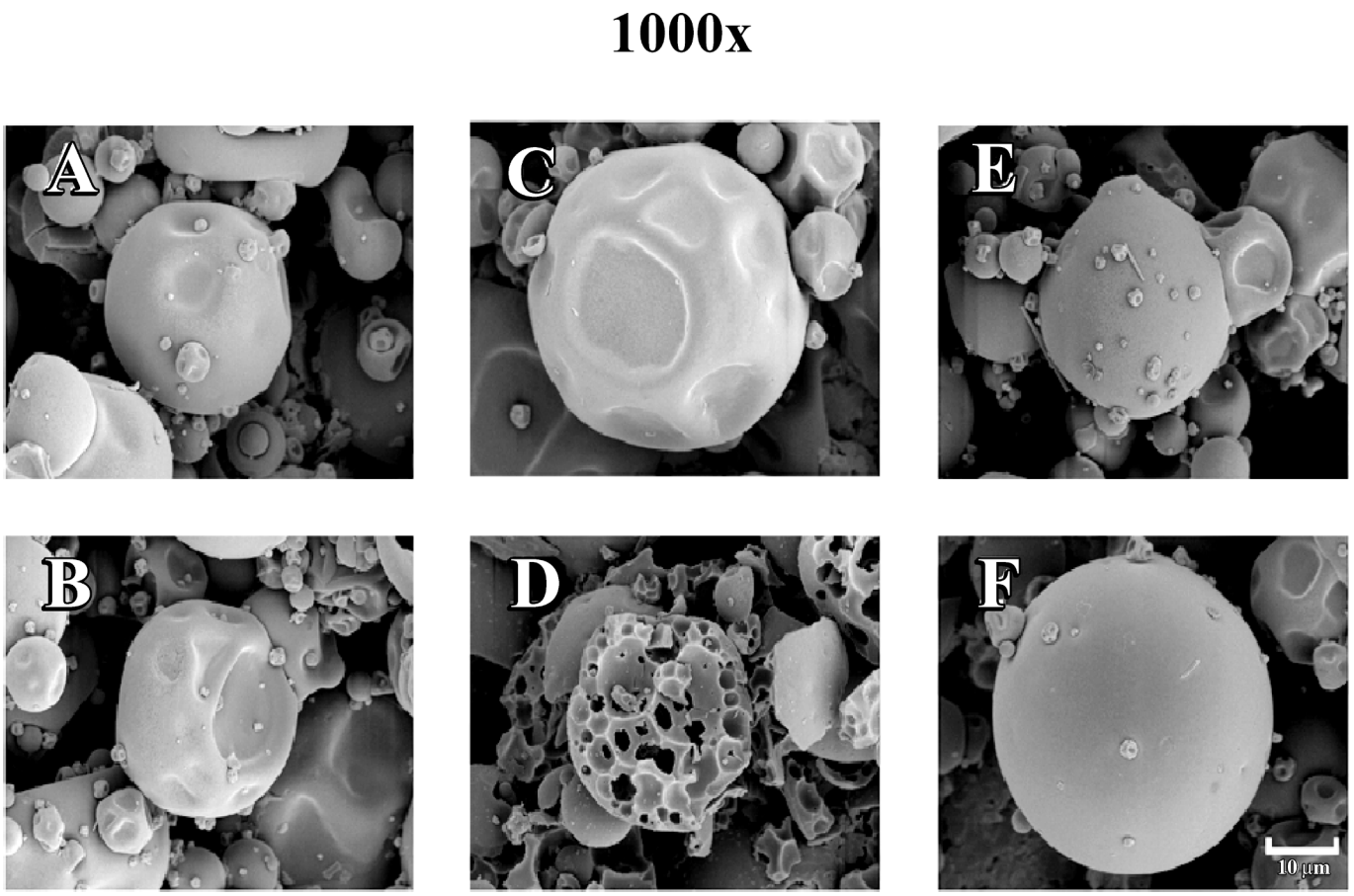

Figure 2. Scanning electron micrographs of the 6 commercial whey protein concentrate with 80% protein products. All products except one, D, show the spherical shape characteristic of a spray-dried whey protein concentrate (Caric, 1994). Products A, B, and C have indentations on the surfaces, typical of whey powders spray dried with low-capacity nozzles (Caric, 1994). The surface of particles of products E and F are smooth, typical of powders spray dried with high-capacity nozzles. The atypical product, D, showed evidence of a powder that was first spray-dried with a high-capacity nozzle, and then milled, revealing fine particles with internal voids and crevices.

RESULTS

The samples contained between 74 and 78% protein (Table 1). Physical functional properties such as protein solubility, amount of denatured protein, foaming and foam stability, and gel strength were determined for the WPC80 products and are reported in Table 2 and Figure 1.

The scanning electron micrographs of the surface of the 6 commercial WPC80 products reveal a wide variation in surface structure based on the method of manufacture (Figure 2). All products except one, D, show the spherical shape characteristic of a spray-dried whey protein concentrate (Caric, 1994). Products A, B, and C have indentations on the surfaces, typical of whey powders spray dried with low-capacity nozzles (Caric, 1994). The surface of particles of products E and F are smooth, typical of powders spray dried with high-capacity nozzles. The atypical product, D, showed evidence of a powder that was first spray-dried with a high-capacity nozzle, and then milled, revealing fine particles with internal voids and crevices.

Proximate composition of the 6 WPC80 samples shows a wide and significant ($P < 0.05$) variation in moisture, protein, fat, ash, and carbohydrate content (Table 1). Physical properties, particle sizes, and density varied widely (Figure 1). Also, all functional properties—solubility, gel strength, foam volume, and stability—varied significantly ($P < 0.05$). Some definite trends or associations were identified, which show the correlation of particle sizes with proximate composition and functional properties. Product D was the most unique of all and had a uniformly distributed small size with mean size of 50 microns. It was observed that smaller particle size correlated with lower fat content and higher solubility. There was no definite association of particle with a trend in either gel

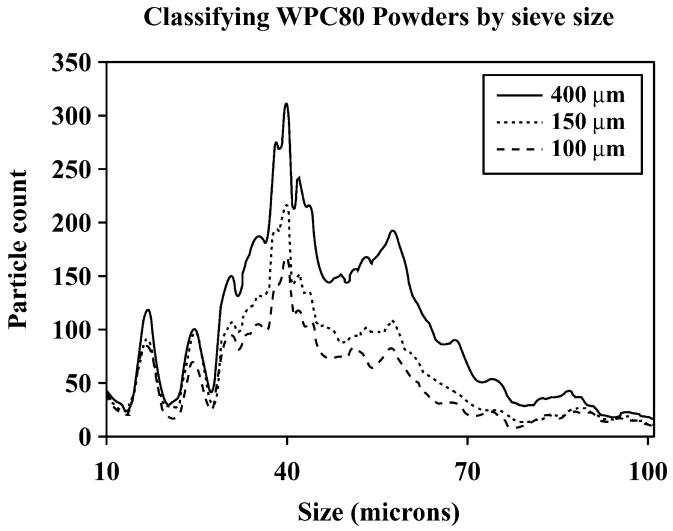

Figure 3. Particle size distribution of whey protein concentrate with 80% protein (WPC80) samples "as purchased," 100 mesh and 140 mesh.

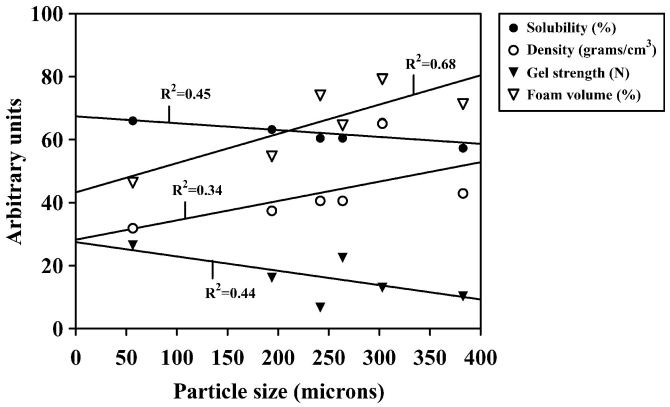

Figure 4. Physical properties of whey protein concentrate with 80% protein 100 mesh samples: solubility, density, gel strength, and foam volume.

strength, foam volume, or stability; these varied from product to product.

Sieving each of the 6 WPC80 products resulted in 2 subsets for each product with typical distribution patterns (Figure 3). The size distributions were layered from the largest size on top to the smallest on the bottom. The top is the "as purchased" sample with all sizes; the second layer, the 100-mesh powders in the range of 100 to 150 microns; at the bottom, the 140-mesh products with sizes below 100 microns.

Proximate composition analysis of the 6 WPC80 100-mesh powders, in the range of 100 to 150 microns, still show significant ($P < 0.05$) variation in moisture, protein, ash, and carbohydrate, but no longer in fat content (Table 2). Density did not vary. The functional properties, solubility and foam volume were now more uniform with a smaller spread in the values. Solubility spread in the "as purchased" values, Table 2, was 37

Table 3. Proximate composition and physical properties of the whey protein concentrate with 80% protein (140 mesh).[1]

Product	Moisture %	Protein %	Fat %	Ash %	Carbohydrate %
A	4.4[a]	73.6[c]	1.6	2.6[c]	17.8[b]
B	4.0[ab]	79.1[b]	3.5	3.1[bc]	10.3[cd]
C	4.3[a]	80.0[a]	2.9	2.5[c]	8.3[d]
D	3.7[bc]	73.4[c]	1.9	3.2[b]	20.8[a]
E	3.5[c]	79.9[b]	2.8	4.5[a]	9.3[d]
F	4.2[a]	75.0[bc]	3.2	4.8[a]	12.8[c]

[a,b,c,d]Means within a column with the same letter are not significantly different.

[1]Particle size: less than 100 microns. Means within a column with the same letter are not significantly different.

SAS covariance factor loading, but after sieving it dropped to 12 points. The drop in value indicate a pulling together of the mean values for functional properties (Figure 4). This drop in covariance loading is associated with a more uniform particle size distribution. This drop is seen also in the loss of correlation of functional properties with particle size. The same trend was obtained with the 6 WPC80 100-mesh powders, in the range of 100 to 150 microns, was observed with the 6 WPC80 140-mesh powders, in the particle size range less than 100 microns (Table 3). The difference was a slightly higher factor loading of 20 for solubility (Figure 5). This shows that WPC80 products in the range of 100 to 150 microns have uniform functionality, their obviously different manufacturing processes notwithstanding.

The pasting viscosity profiles of the various products show differences based on the source of the WPC80

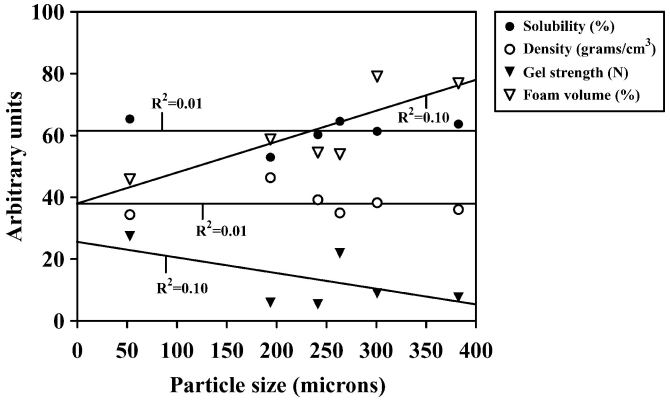

Figure 5. Physical properties of whey protein concentrate with 80% protein 140 mesh: solubility, density, gel strength and foam volume.

Table 4. Rapid Visco-Analyzer pasting properties of whey protein concentrate with 80% protein.[1]

	Peak viscosity (cP)						Final viscosity (cP)					
Product	A	B	C	D	E	F	A	B	C	D	E	F
As is	209	191	178	270	141	248	616	544	379	976	667	575
100	140	179	154	294	52	174	369	589	492	993	163	660
140	182	177	126	239	29	81	520	537	397	983	54	381

[1]Pooled standard deviation: peak viscosity = 38; final viscosity 296.

(Table 4). On this typical viscosity profile for WPC80 (Figure 6), points of interest are shown, for peak and final viscosity. The least particle sized WPC80 product, D, had the highest peak and final viscosity of all 6 products and was consistently higher than the others in the smaller fractions (Table 4). Using the pooled standard deviation as an indicator, product D, was significantly different from all other products. Pooled pasting property for each mesh size show the effect of particle size on the pasting pattern. The "as purchased" samples were consistently higher than the 100-mesh and 140-mesh pooled samples in peak and final viscosity (Figure 7).

Overall SAS covariance factor-loading estimate for the "as purchased" WPC80 products was 252, indicating a wide variation, but with the 100-mesh products the loading factor dropped to 11, indicating much less variance, but for the 140-mesh powders, the loading factor was 20. This indicates that the 100-mesh powders had a more uniform proximate, physical, and functional characteristics than the "as purchased" products or the 140-mesh products.

DISCUSSION

The properties of any food particulate system are primarily dependent on its particle size distribution (Yan and Barbosa-Canovas, 1997). Based primarily on the sizes of WPC80 products, there were significant ($P < 0.05$) variations in size, shape, and density. The fat content, solubility, gel strength, and foaming properties correlated with the particle size distribution. Regester et al. (1992), in a survey of 6 commercial whey protein concentrates, with approximately 34% protein, reported considerable variability in chemical composition, ash, pH, solubility, and digestibility attributed mostly to product source and processing. Our samples contained 80% protein and exhibited similar differences, but the results indicate that differences are largely associated with the particle sizes of the products.

The functional properties of whey protein concentrates are influenced largely by their chemical and physical properties, and these properties in turn are influenced by the conditions of cheese manufacturing and whey powder processing (Hawks et al., 1993). The preparations are highly variable because of many different processes that are used by different manufacturers (Schmidt et al., 1984). Although there are many variables affecting the chemical properties of whey protein concentrate from the farm through cheese manufacturing, the major process variable that affects functionality is related to the extent of protein denaturation during processing. The level of insoluble de-

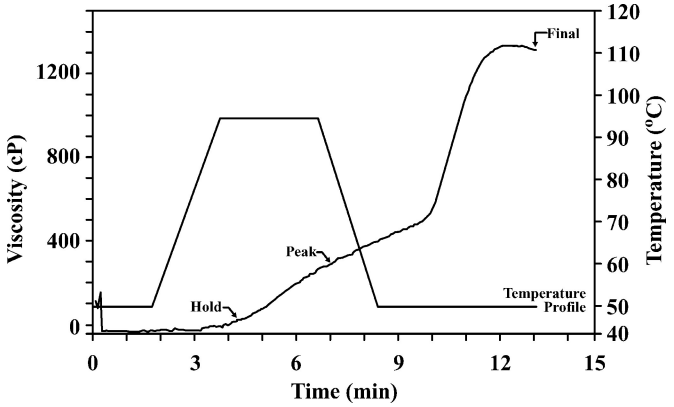

Figure 6. Schematic illustration of a typical Rapid Viscosity Analyzer of whey protein concentrate with 80% protein showing peak and final viscosity.

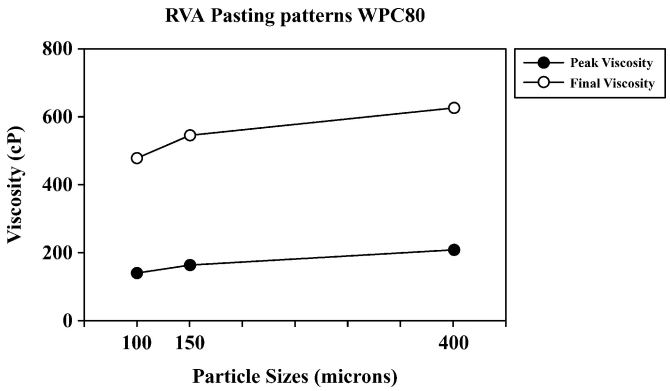

Figure 7. Rapid Viscosity Analyzer (RVA) pasting patterns of whey protein concentrate with 80% protein samples "as purchased," 100 mesh, and 140 mesh.

natured proteins in WPC products affect solubility, and solubility determines functionality (Schmidt et al., 1984; Puyol et al., 1999). In analyzing the properties of the WPC80 purchased, we see that the same trend holds. Therefore, removing the insoluble denatured proteins will always improve functionality, despite the product history or the way it was processed.

Some insoluble partially denatured proteins can be reversed, making them more soluble. Reversible changes occur mostly below 60°C; irreversible changes occur above 100°C, affecting solubility mostly (deWit and Klarenbeek, 1983). Variations in solubility in "as purchased" WPC80 products (Table 1), reflects the extent of heat treatment. The differences were significant ($P < 0.05$), predicating differences in functionality, which were seen in gel strength, foam volume, and stability. Processing methods used in manufacturing whey concentrates do alter the property of the proteins (denature), particularly the highly refined concentrates with protein content over 34%, which undergo special separation and filtration processes (Huffman and Harper, 1999). Further drying to produce WPC powder causes more denaturation, which is the cause of loss of solubility (deWit and Klarenbeek, 1983).

Other source- and process-dependent variability in functional properties such as in gel strength and foaming occur due to the cheese making procedure. Whey is the product of cheese manufacturing. Various methods are used to precipitate casein from milk leaving the whey proteins in solution, which are concentrated into WPC of different protein contents. Solubility of whey proteins is at pH 6.2 or above at the point casein is precipitated for cheese making. deWit and Klarenbeek (1983) have shown a pH-dependent effect on solubility. So, the solubility of a given WPC product is also affected by the pH history of the cheese source.

Foaming of whey protein concentrate is affected by solubility and the amount of residual lipid in the product (Hawks et al., 1993). By filtering the whey protein concentrate slurry to remove large molecular mass components, Hawks et al. (1993) improved the foaming property of whey protein concentrate samples. We have also observed that removing large particles by sieving redistributes the amount of fat present in the WPC80 products improved foam volume, particularly in the smallest particle size fractions (Table 3).

Sieving to remove the large particles improved solubility significantly, indicating that the large particles are more denatured and consequently more insoluble. Any method used to reduce the size of the whey protein concentrates will improve their functionality.

CONCLUSIONS

There were wide variations in properties of the 6 WPC80 purchased from commercial sources, but the variations in functionality were minimized through sieving and using powders with a smaller particle size distribution range. Significant reduction in particle size correlated with improved solubility of the products. Sieving the WPC80 and using powders in the range of 100 to 150 microns resulted in a more uniform functionality. The most soluble fractions had particles less than 100 microns.

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